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# Preferential DNA Repair in Expressed Genes

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**Potentially deleterious alterations to DNA occur nonrandomly within the mammalian genome. These alterations include the adducts produced by many chemical carcinogens, but not the UV-induced cyclobutane pyrimidine dimer, which may be an exception. Recent studies in our laboratory have shown that the excision repair of pyrimidine dimers and certain other lesions is nonrandom in the mammalian genome, exhibiting a distinct preference for actively transcribed DNA sequences. An important consequence of this fact is that mutagenesis and carcinogenesis may be determined in part by the activities of the relevant genes. Repair may also be processive, and a model is proposed in which excision repair is coupled to transcription at the nuclear matrix. Similar but freely diffusing repair complexes may account for the lower overall repair efficiencies in the silent domains of the genome.**

**Risk assessment in relation to chemical carcinogenesis requires assays that determine effective levels of DNA damage for producing malignancy. The existence of nonrandom repair in the genome casts into doubt the reliability of overall indicators of DNA binding and lesion repair for such determinations. Furthermore, some apparent differences between the intragenomic repair heterogeneity in rodent cells and that in human cells mandate a reevaluation of rodent test systems for human risk assessment. Tissue-specific and cell-specific differences in the coordinate regulation of gene expression and DNA repair may account for corresponding differences in the carcinogenic response.**

## Introduction

In studying the complex relationships between the repair of damaged DNA in mammalian cells and biological end points such as survival, mutagenesis, and transformation, the repair proficiency has usually been assumed to be uniform throughout the genome and even within cell populations. However, we now know that DNA damage in some regions of the genome is processed much more efficiently than that in others (*1,2*). Heterogeneity in repair would result in corresponding differences in the responses seen for particular biological effects, since the consequences of unrepaired or misrepaired damage in DNA clearly depend upon the precise location of the damage with respect to the relevant domains in the genome. At the nucleotide sequence level in bacteria this fact is well documented by correlations of the spectrum of particular lesions with hot spots for mutagenesis. Similar information on the specificity of the mutagenic response in relation to the spectrum of DNA damage is now being obtained in mammalian systems and is being analyzed at the various levels of genomic organization. Differences in the repair response to damage in selected genomic regions may account for some of the profound differences seen in the carcinogenic response in different tissues or when different organisms are compared. It is therefore essential to appreciate the rules governing the fine structure of DNA repair in mammalian genomes in order to assess carcinogenic risks.

Most methods used to study DNA repair are not intrinsically sensitive to intragenomic heterogeneity in the distribution of repair events. Measurement of repair replication by the 5-bromodeoxyuridine density-labeling protocol (*3*) is insensitive to such heterogeneity, as is the direct determination of adduct release from genomic DNA. However, the methods that use lesion-specific endeonucleases can, in principle, be used to detect heterogeneities in DNA repair within a population of DNA molecules (*4*). Thus, any DNA molecules selectively repaired should retain their integrity upon exposure to such endonucleases, while those molecules inaccessible to repair will remain sensitive to cleavage at the sites of the persisting lesions. This approach was used by Mansbridge and Hanawalt (*5*) to demonstrate intracellular heterogeneity in the efficiency of repair of UV-induced cyclobutane pyrimidine dimers in fibroblasts from patients with xeroderma pigmentosum, complementation group C.

An early indication of DNA repair heterogeneity was the observation of a biphasic time course for the removal of DNA lesions in cultured mammalian cells (*6*) and in rodents *in vivo* (*7–10*). In cultured human cells the rapid removal of pyrimidine dimers in the first few hours after

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UV irradiation was followed by a gradually decreasing rate, approaching a plateau by 24 hr. Two different rates of pyrimidine dimer removal were noted by Kantor and Setlow (*11*), who interpreted their results as an indication of different classes of accessibility to repair enzymes. Also consistent with repair heterogeneity was the observation of rapid recovery of RNA synthesis in UV-irradiated mammalian cells, which led to the suggestion of preferential repair in transcriptionally active chromatin (*12*).

There have been a number of attempts to determine DNA damage and repair levels in expressed genes in mammalian cells. Thus, evidence was obtained by Arrand and Murray (*13*) for selective binding and removal of benzo[a]pyrene adducts in the active chromatin (i.e., DNAse I sensitive) of the genome. The production of alkaline labile sites by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine in a 4-kb fragment of the collagen gene and in a 5-kb fragment of the β-globulin gene in contact inhibited human WI38 cells (*14*). These authors reported roughly similar rates of disappearance of intact restriction fragments analyzed on alkaline gels with increasing dose of carcinogen. The change in the number of alkaline labile sites with time after treatment also appeared similar for the two fragments investigated, but it was not known which particular lesions were being studied. It is likely that the repair being monitored in this analysis was initiated by the glycosylase-AP endonuclease pathway rather than by the general nucleotide excision-repair pathway.

## Selective Repair of Pyrimidine Dimers in an Active Gene

We have recently quantified the levels of cyclobutane pyrimidine dimers in the vital dihydrofolate reductase (DHFR) gene of UV-irradiated Chinese hamster ovary (CHO) cells using the bacteriophage T4 endonuclease V as a sensitive probe for dimers (*15*). Restricted genomic DNA was treated with and without T4 endonuclease, electrophoresed under denaturing conditions, transferred to nitrocellulose paper, and then hybridized to a $^{32}$P-labeled probe specific for a restriction fragment in the gene. The proportion of fragments free of endonuclease sensitive sites in each sample was determined from the difference in the amount of probe hybridized at the position of full-length fragments for T4 endonuclease-treated and untreated samples. Use of a cell line in which the DHFR gene was amplified roughly 50-fold helped greatly with the sensitivity of the analysis.

Although 70% of the pyrimidine dimers were removed from a restriction fragment within the DHFR gene, little repair was detectable in a fragment 30 kb upstream from the gene, and only 15% of the dimers were lost from the genome overall in 24 hr. These results were confirmed in CHO cells containing only a few copies of the DHFR gene, so the observed microheterogeneity in repair is clearly unrelated to the phenomenon of gene amplification (*16*). Rodent cell lines typically exhibit UV resistance similar to that of repair-proficient human cell lines, but carry out much lower levels of repair (*17*). On the basis of our results, we suggested that the preferential repair of vital DNA sequences accounts for the high UV resistance of the CHO cells, in spite of their low overall repair levels. Survival of cells after exposure to UV thus correlates with repair in an essential gene rather than with overall genomic repair. Although this is a plausible explanation for the rodent versus human cell paradox, it leaves unanswered the important question of how the replication machinery is able to overcome the many persisting lesions in the rodent cell DNA to generate functional daughter genomes. This problem is discussed later.

In our analyses it was essential to determine the contribution of semiconservative replication to the appearance of dimer-free DNA in the restriction fragment bands in the gels. The addition of hydroxyurea to the cell cultures to suppress replicative DNA synthesis did not appreciably affect the apparent level of repair, and therefore the generation of dimer-free DNA must have been mostly due to repair (*15*). The small amount of replicated DNA was categorically eliminated in some experiments, in which 5-bromodeoxyuridine was included in the growth medium after irradiation, to density-label the replicated DNA in the cultures. Following enzymatic restriction, the replicted DNA was then separated from the unreplicated DNA on CsCl equilibrium density gradients so that the T4 endonuclease V-sensitive site analysis could be carried out on parental DNA that had not undergone replication. Our results were consistent with those obtained on total DNA (*15*). This general approach also provided a means to follow the fate of dimers (or other lesions) in both unreplicated and in replicated DNA in the same experiment.

In contrast to the results with CHO cells, the repair analysis for pyrimidine dimers in human cells in which the DHFR gene is amplified did not reveal significant differences in the extent of repair within the gene and in nearby sequences. Of course, normal human cells are repair proficient overall for pyrimidine dimers, so one would not expect to find striking differences in repair in the various genomic domains. However, a careful analysis of the time course of repair indicated that pyrimidine dimers in the DHFR region were repaired earlier than were those in the nontranscribed α-DNA sequences or in the bulk of the genome (*18*).

Our demonstration of selective repair in the DHFR gene in both CHO and human cell lines raised questions about the nature and extent of the repair domain. We were able to effectively map the DNA repair domain in the DHFR region in CHO cells by analysis of the efficiency of pyrimidine dimer removal from a series of overlapping restriction fragments, 7 to 30 kb in length, which spanned the entire gene and its surrounding sequences (*19*). We found that within 8 hr after a UV dose of 20 J/m$^2$, the cells had removed more than 40%

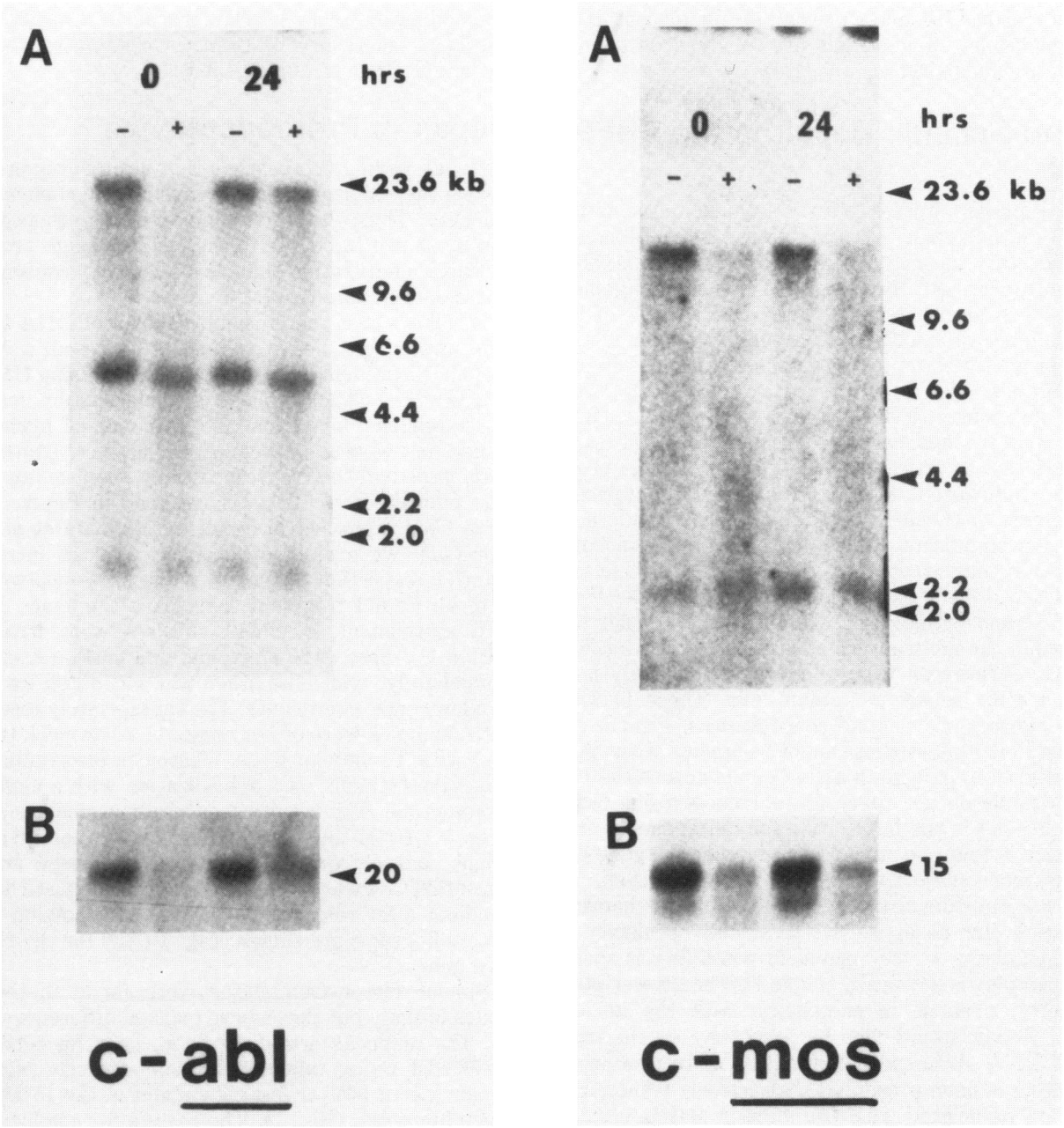


FIGURE 1. Comparative repair of pyrimidine dimers in an active and an inactive protooncogene. The autoradiograms show the analysis of T4 endonuclease sensitive sites in the 20 kb intragenic BamH1 restriction fragment of c-*abl* immediately after UV irradiation of the confluent mouse cells and 24 hr later as described in the text (*A*). Similarly, the 15 kb EcoR1 fragment spanning the c-*mos* locus was analyzed in the same experiment (*B*). Samples treated with T4 endonuclease are indicated by (+) and those not treated with T4 by (−). In a second experiment, the analysis was carried out on parental DNA isolated in CsCl gradients from actively growing cells and the same fragments were analyzed for c-*abl* and c-*mos*, respectively (*C*,*D*). Reproduced by permission from Cell Press (*32*).

of the dimers from sequences near the 5′ end of the gene, somewhat fewer from the 3′ end, but almost none from flanking sequences at the 3′ end or upstream of the promoter region. On the basis of these results we have estimated that the region of preferential repair at the DHFR locus extends over 50 to 80 kb. We used isoschizomeric restriction enzyme analysis with Msp I and Hpa II to detect the level of methylated cytosine in CCGG sequences within the same fragments used for the repair analysis. Interestingly, we found hypomethylated sites only in the 5′ end fragment that displayed the highest repair efficiency (*19*). The reduction in the level of repair toward the 3′ end of the gene could reflect a processive mode of the repair enzyme complex. Pro-

cessivity of the T4 endonuclease on dimer-containing DNA molecules *in vitro* has previously been demonstrated in our laboratory (*20,21*).

## Strand-Specific Repair in the DHFR Gene

In preliminary studies to further elucidate the nature of preferential repair in active genes, Isabel Mellon of our laboratory has compared repair in the transcribed and nontranscribed DNA strands in the DHFR gene in CHO and in human cells. A genomic fragment of the DHFR gene was cloned into a vector containing two phage promoters oriented in opposing directions so that RNA probes could be produced to quantify the transcribed and nontranscribed strands, respectively. These analyses were then carried out in turn on the same Southern blot to which the nick-translated DNA probe had been hybridized. Although the same level of pyrimidine dimers was measured initially in each strand, there was a very significant difference between the efficiency of repair in the two strands. In the CHO cells, nearly 80% of the dimers had been removed from the transcribed strand within 4 hr, while almost no repair had occurred in the nontranscribed strand. Similar but less dramatic differences were evident in the analysis of strand-specific repair in human cells. These findings have a number of profound consequences. First is the fact that Poisson statistics cannot be applied with validity to analyze repair in an active gene because the two DNA strands do not represent a homogeneous population. Second is the implication for mutagenesis—the prediction is that one strand in a given active gene will be much more subject to mutation than the other.

One can consider a number of possible mechanisms to achieve the observed DNA strand specificity in repair. Of these, a very plausible possibility is that a repair complex is physically coupled to the transcription machinery, perhaps in association with the nuclear matrix. Repair would then be processive on the transcribed DNA strand in the direction of transcription. Evidence has been presented that actively transcribed genes are associated with the nuclear matrix (*22,23*), and some recent studies have implicated the nuclear matrix in DNA repair (*24,25*), although others find this localization only in cells from xeroderma pigmentosum complementation group C (*26*). The transcription-coupled repair complex would serve to clear bulky, transcription-blocking adducts from active genes selectively. To account for the repair that occurs in nontranscribed regions of the genome (and in the nontranscribed strands of active genes), one could postulate additional, freely diffusing repair complexes. A prediction of this model is that mutants might be found in which either of the respective repair modes could be defective. In Cockayne's syndrome the characteristic cellular UV sensitivity is accompanied by an evident defect in the rapid resumption of RNA synthesis in the irradiated cells (*27,28*). In preliminary studies a corresponding defect in selective repair of an active gene has been noted in cells from a Cockayne's syndrome patient (I. Mellon, unpublished).

## Repair in Protooncogenes

It has been hypothesized that protooncogenes are among the cellular targets of physical and chemical carcinogens. Thus, the activation of H-*ras* protooncogenes can be shown to be due to the DNA damage produced by carcinogens rather than as a secondary consequence of transformation (*29–31*).

It follows that the efficient removal of DNA lesions from protooncogenes at risk could represent a critical step in the prevention of tumorigenesis. Using UV-irradiated mouse 3T3 fibroblasts as model system, we have compared the formation and removal of pyrimidine dimers in two protooncogenes, the actively transcribed c-*abl* gene and the transcriptionally silent c-*mos* gene. The published results are illustrated in Figure 1 and have been discussed in detail by Madhani et al. (*32*). For c-*abl* we examined repair in a 20 kb intragenic BamH1 restriction fragment, while for c-*mos* we used a 15 kb EcoR1 fragment that spans the locus. In the first experiment, confluent cultures were irradiated with a UV dose of 20 J/m$^2$, and one portion was lysed immediately, while the other was incubated for 24 hr to allow repair before lysis. The appropriately restricted DNA samples were either treated (+) or mock treated (−) with T4 endonuclease V prior to electrophoresis, Southern transfer, and hybridization with a c-*abl* or a c-*mos* probe. The resultant autoradiograms are shown (Fig. 1 *A,B*) for the respective genes. In a second experiment, actively growing cultures were used and the parental DNA was isolated for analysis in a CsCl density gradient after restriction. The relevant portion of the autoradiograms are shown (Fig. 1 *C,D*) for the respective genes.

Similar dimer frequencies were found in the two genes initially but there were marked differences by 24 hr. The reappearance of the 20 kb band for c-*abl* indicates that repair (about 85%) has occurred, but little repair (about 20% or less) is evident in the 15 kb band spanning c-*mos* (Fig. 1). The results for confluent and for actively growing cell cultures are similar and lead to the general prediction that more UV-induced mutations should accumulate in the c-*mos* region and in other silent domains than in the c-*abl* gene in these cells. While one cannot draw conclusions about the specific role of protooncogene repair in relation to tumorigenesis from this study, it provides a paradigm for similar analyses when the genes at risk in a particular tissue are eventually known.

## Repair and Replication of a Gene Carrying Chemical Adducts

As discussed above, UV-induced pyrimidine dimers are distributed uniformly in active and inactive genomic domains. However, most carcinogens are chemicals,

and the DNA adducts produced are generally distributed nonrandomly in chromatin (*33*). Therefore, it is important to study the introduction and repair of chemical carcinogen adducts in specific DNA sequences. Our earlier analysis of DNA repair in confluent African green monkey kidney cells had revealed a deficiency in repair of certain bulky chemical adducts (including psoralens, aflatoxin $B_1$, and *N*-acetoxy-acetyl aminofluorene) in the nontranscribed α-DNA sequences compared to that in the bulk DNA (*34,35*). As noted earlier, the repair of pyrimidine dimers proceeds at similar rates in α and bulk DNA (*18,34*).

We have now examined DNA repair in an active gene in cultured human cells treated with the psoralen derivative 4′-hydroxymethyl-4,5′,8-trimethylpsoralen (HMT), using a newly developed sensitive assay for interstrand DNA cross-linking in defined genomic sequences (*36*). Within 24 hr, 80% of the cross-links, but only 45% of the monoadducts, were removed from a 32 kb transcribed sequence in the DHFR gene, demonstrating that the repair efficiency in an active human gene is dependent upon the nature of the damage. HMT monoadducts were also detected in the replicated DNA of the DHFR gene at frequencies nearly as high as in the parental DNA, indicating that such adducts may not greatly interfere with replication. Furthermore, the existence of cross-linkable monoadduct sites in the replicated DNA implies that the newly synthesized strand is continuous opposite those sites and that the bypass mechanism is relatively error-free. Base changes in the daughter strand opposite the monoadduct site would generally be expected to preclude the formation of cross-links, as cross-links require appropriately positioned pyrimidines. We have suggested that translesion DNA synthesis could be another mechanism, in addition to strand-specific repair, to circumvent transcription blockage in a damaged gene, as the duplicate copy could then be used as a hindrance-free template for transcription (*36*).

Our analysis of the persistence of bulky chemical adducts in defined sequences (*35,36*) and the discovery that repair is selective for the transcribed strand in an active gene (Mellon et al., submitted for publication) strongly support the view that while most bulky lesions in DNA pose no insurmountable problems for replication, they must be removed from essential transcribed sequences to maintain viability. Bulky lesions such as pyrimidine dimers block transcription (*37*), so the presence of one or more dimers in each copy of an essential gene would be expected to be lethal to the cell. The survival of cells carrying persisting lesions in their DNA implies that those remaining lesions are not in essential genes, but that the lesion containing regions do not prevent genomic duplication.

## Relevance of Preferential DNA Repair to Risk Assessment

For purposes of risk assessment, it is sometimes adequate to measure parameters that are really indicators of exposure level rather than directly related to the potentially deleterious consequences of the exposure. However, such gross determination cannot readily take into account important individual variations that may vastly increase the risks. Therefore, it is important to learn what factors are significant in the probability of progression from exposure to eventual tumor. The binding of carcinogenic compounds in tissues does not always correlate with the tumorigenicity in those tissues, and a very plausible explanation may be that different genes are at risk in the different tissues because of the different respective patterns of gene expression and DNA repair.

It is now clear that the repairability of damage in mammalian chromatin depends upon the type of lesion, its precise location in the genome, and the functional state of the DNA at that particular site. Information obtained on the processing of damage overall or in one domain of the genome may not be relevant to an understanding of a biological response that is dependent upon damage and/or repair activity in another domain. Thus, the question of whether protooncogenes are located in efficiently repairable domains in the tissue at risk may have significance for risk assessment. Since rodents are used widely in carcinogen testing for human risk assessment, it is imperative that we learn the unique features of DNA damage processing in the respective systems. It has been reported that levels of DNA repair in mouse embryo cells decline with successive passage in culture (*38,39*) and also when they are transformed to established heteroploid cultures (*40*). Do some domains become selectively excluded from repair, and if so, which? Do similar changes in DNA repair occur with development and/or aging in the cells *in vivo?* How does the spectrum of proficiently repaired genes and/or domains vary from tissue to tissue in the same organism? Do mutagenic lesions accumulate in underrepaired domains in rodent and human cell genomes? These and many other questions need to be answered in order to understand how the risks of malignant transformation may be related to the fine structure of DNA damage processing in mammalian cells and tissues.

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